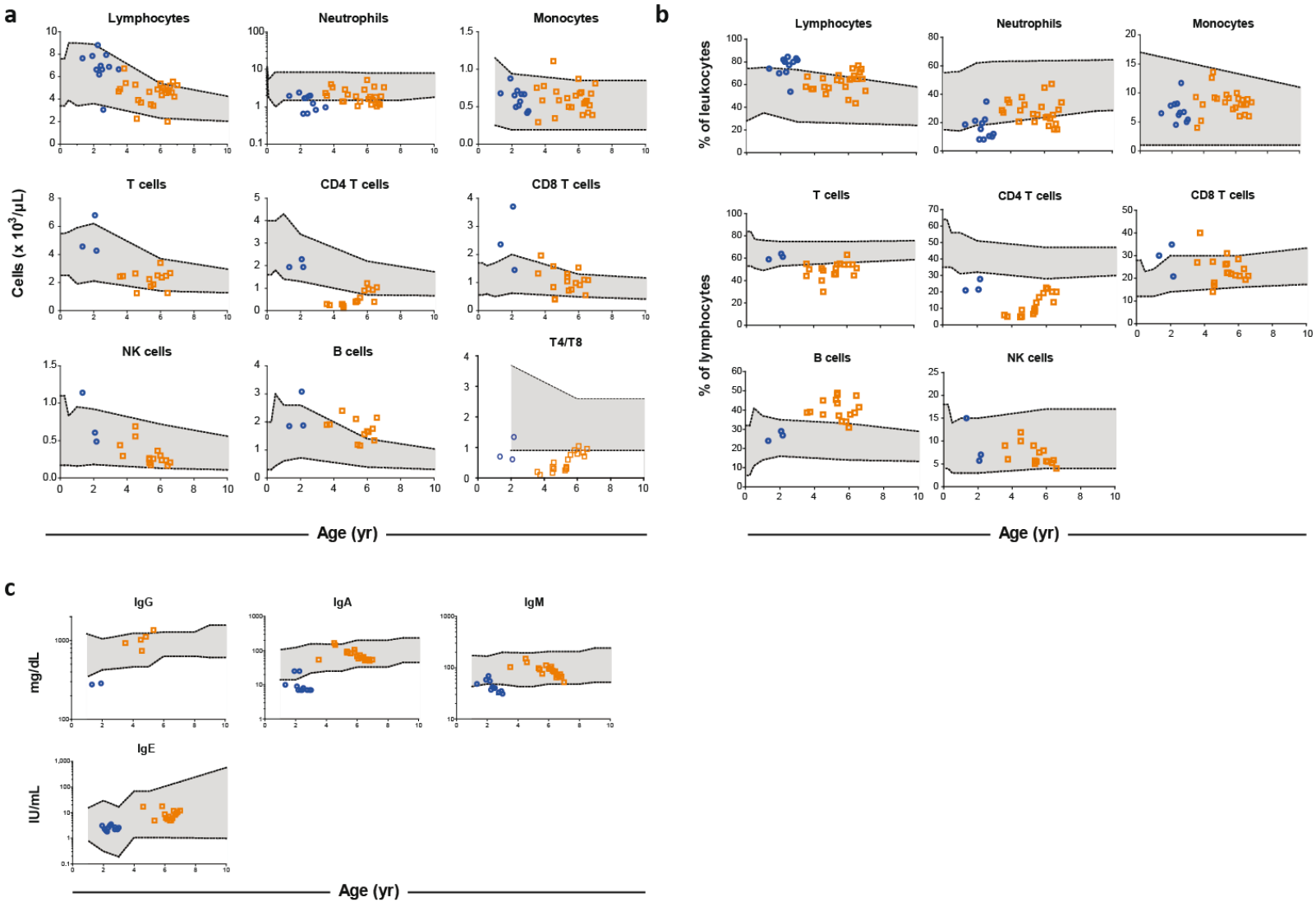
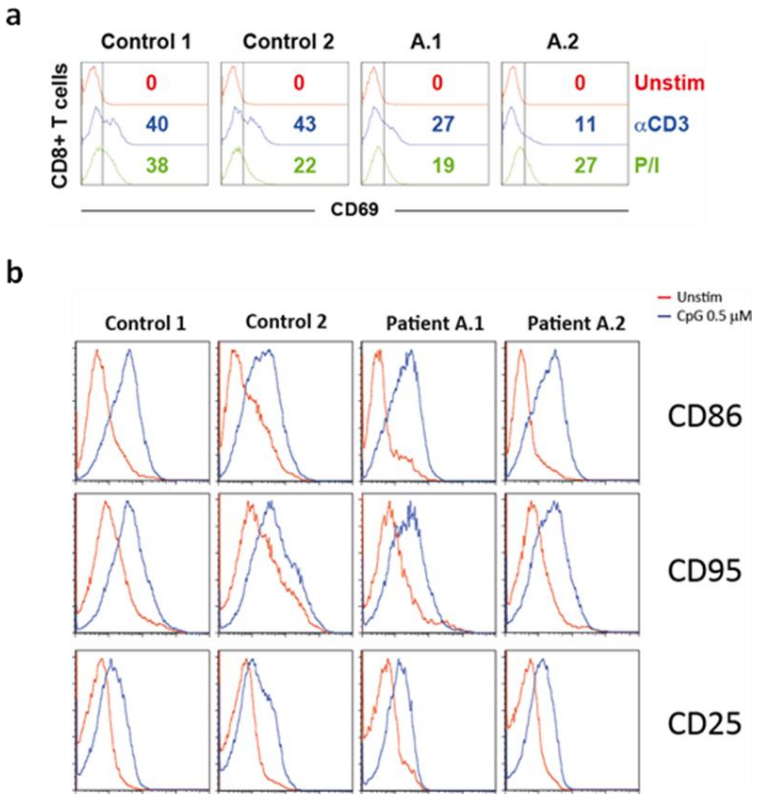
Supplementary Table 1: Clinical phenotype of patients studied

Subject	Patient A.1 (proband)	Patient A.2 (brother)	Patient B.1
Age	7 years old	3 years old	Post mortem (deceased 45 years old)[§]
Mutation	10 bp deletion	10 bp deletion	Non-sense mutation
Clinical history			
Epstein-Barr Virus infection	+	+	+
Herpes simplex virus infection	+	+	−
Viral pneumonia	+	+	−
Otitis media[¶°]	+	+	−
Sinusitis	+	+	−
Chronic diarrhea	+	+	−
Lymphoma	−	−	+
Lymphocyte numbers in peripheral blood (normal ranges for similar age)			
Total lymphocytes (cells/mL)	**1738** (1400-3700)	**4562** (1900-5900)	**285** (460-4700)
CD4 T cells (cells/mL)	**432** (700-2200)	**1935** (1400-4300)	**212** (358-1259)
CD8 T cells (cells/mL)	**717** (490-1300)	**2364** (500-1700)	**25** (194-836)
CD4/CD8 ratio	**0.6** (1.4-1.7)	**0.7** (0.9-3.7)	**8.5** (1.0-3.6)
T regulatory cells (cells/mL)	**41** (25-89)	**91** (25-89)	**ND**
B cells (cells/mL)	**1185** (390-1400)	**1849** (610-2600)	**40** (47-424)
NK cells (cells/mL)	**175** (130-720)	**1139** (160-950)	**5** (87-505)
Serum immunoglobulin level[♭]			
IgG	normal	low	normal
IgA	normal	below detection	normal
IgM	normal	low	low
IgE	normal	normal	ND
Functional antibodies[§]			
Tetanus toxoid	(−)	(+)	ND
Haemophilus influenzae type B, Diptheria	(+), (+)	(+), ND	ND
Pneumococcal	(+)[#]	(+/−)[♮]	ND
T Lymphocyte Activation Function (normal control range)			
% Divided cells* (34%-46%)	**15%**	**21%**	ND
% CD25+ (86%-96%)	**32%**	**54%**	ND
% CD95+ (64%-80%)	**57%**	**39%**	ND
% CTLA-4+ (38%-48%)	**6%**	**12%**	ND

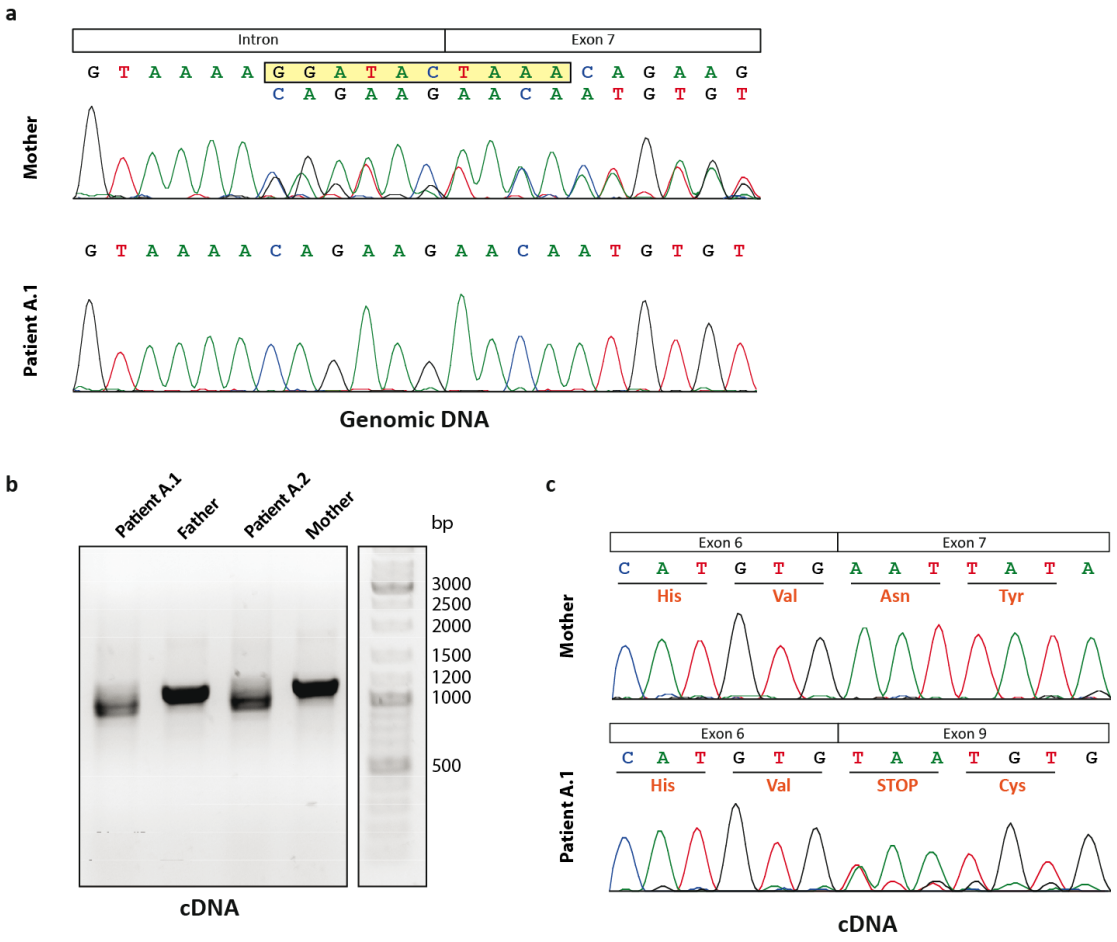
[§] Lymphocyte numbers in peripheral blood were measured a few months prior to death, [¶] with myringtomy tube placement, ° after tonsillectomy & adenoidectomy, [♭] Values are as compared to normal age ranges (see sFig. 1c), ND, not determined (blood could not be obtained from deceased patient), [§] tested after 4 doses of vaccines, [#] positive for 6 of 7 tested serotypes after pneumococcal 7-valent conjugate vaccine, [♮] positive for 3 to 4 of 7 tested serotypes after pneumococcal 7-valent conjugate vaccine, * in CFSE proliferation assay analysed by the Proliferation function in Flowjo



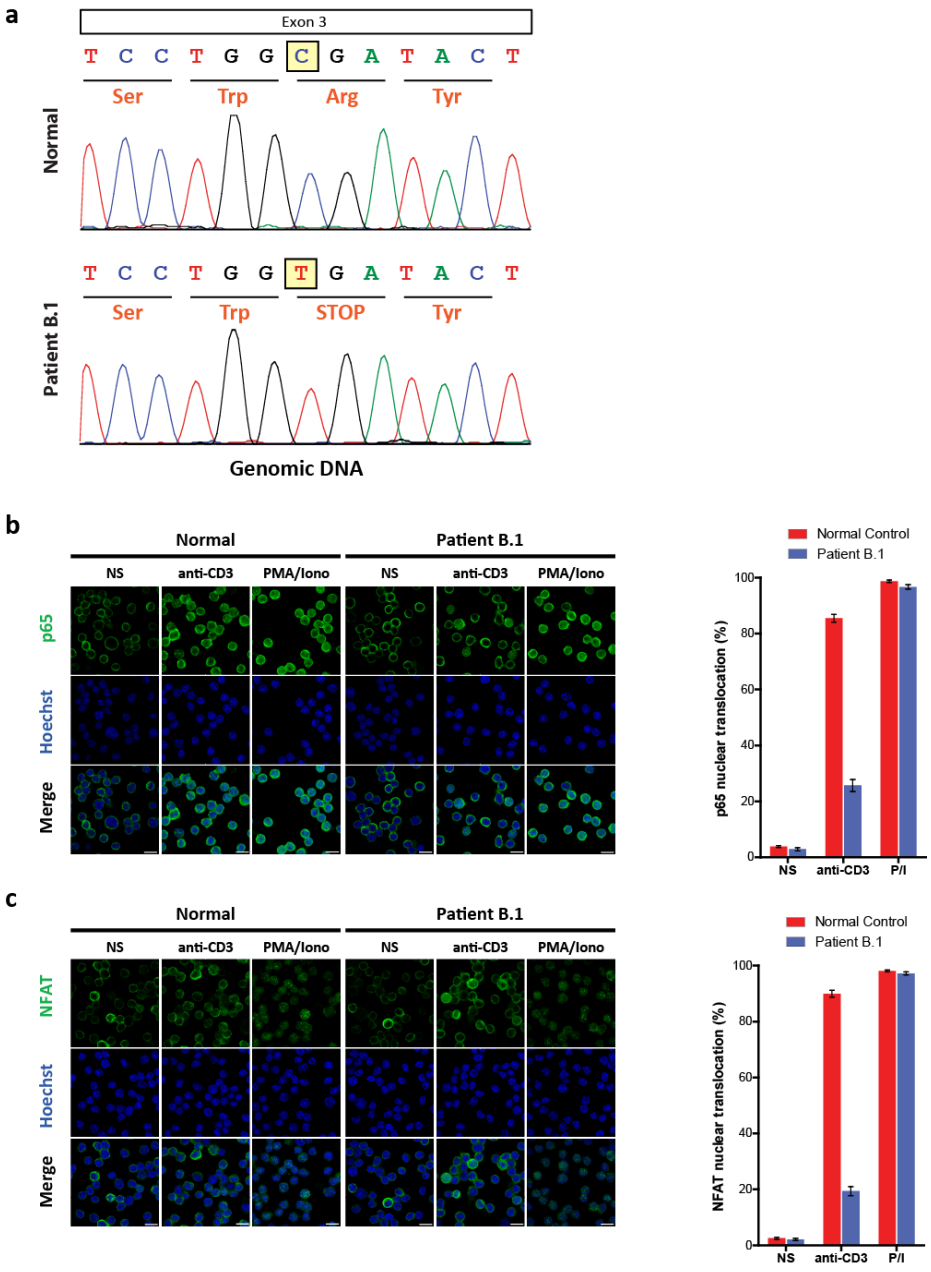
Supplementary Figure 1: ICL patients have abnormally low CD4:CD8 T cell ratio. Absolute cell count (**a)** and relative percentage (**b**) of various immune cell populations and immunoglobulin levels (**c**) documented by clinical flow cytometry labs for patient A.1 (orange squares) and patient A.2 (blue circles) during various hospital admissions. T4/T8 = CD4/CD8 T cell ratio. Shaded grey area represents the range of age-matched normal control values (10^{th} to 90^{th} percentiles for lymphocyte subgroups[52] and 95% confidence interval for serum immunoglobulin[53]).



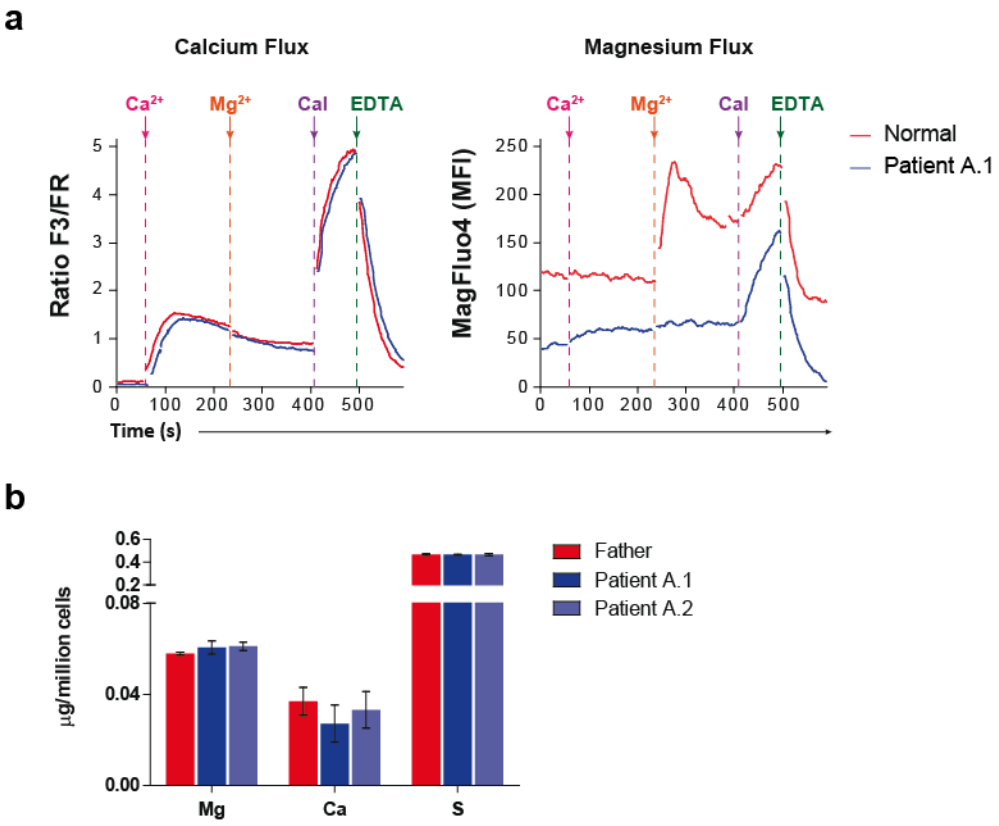
Supplementary Figure 2: Activation of CD8$^+$ T cells and B cells from patients. **a,** CD69 expression in CD8$^+$ cells after stimulation with anti-CD3 (αCD3), PMA/Ionomycin (P/I) or unstimulated (Unstim). Numbers represent percentage CD69$^+$ cells. **b,** CD86, CD95, and CD25 expression in B cells purified from normal controls and patient A.1 and A.2 with (CpG 0.5 μM) or without (Unstim) CpG oligodeoxynucleotide (ODN 2395, InvivoGen) stimulation for 72 hrs.



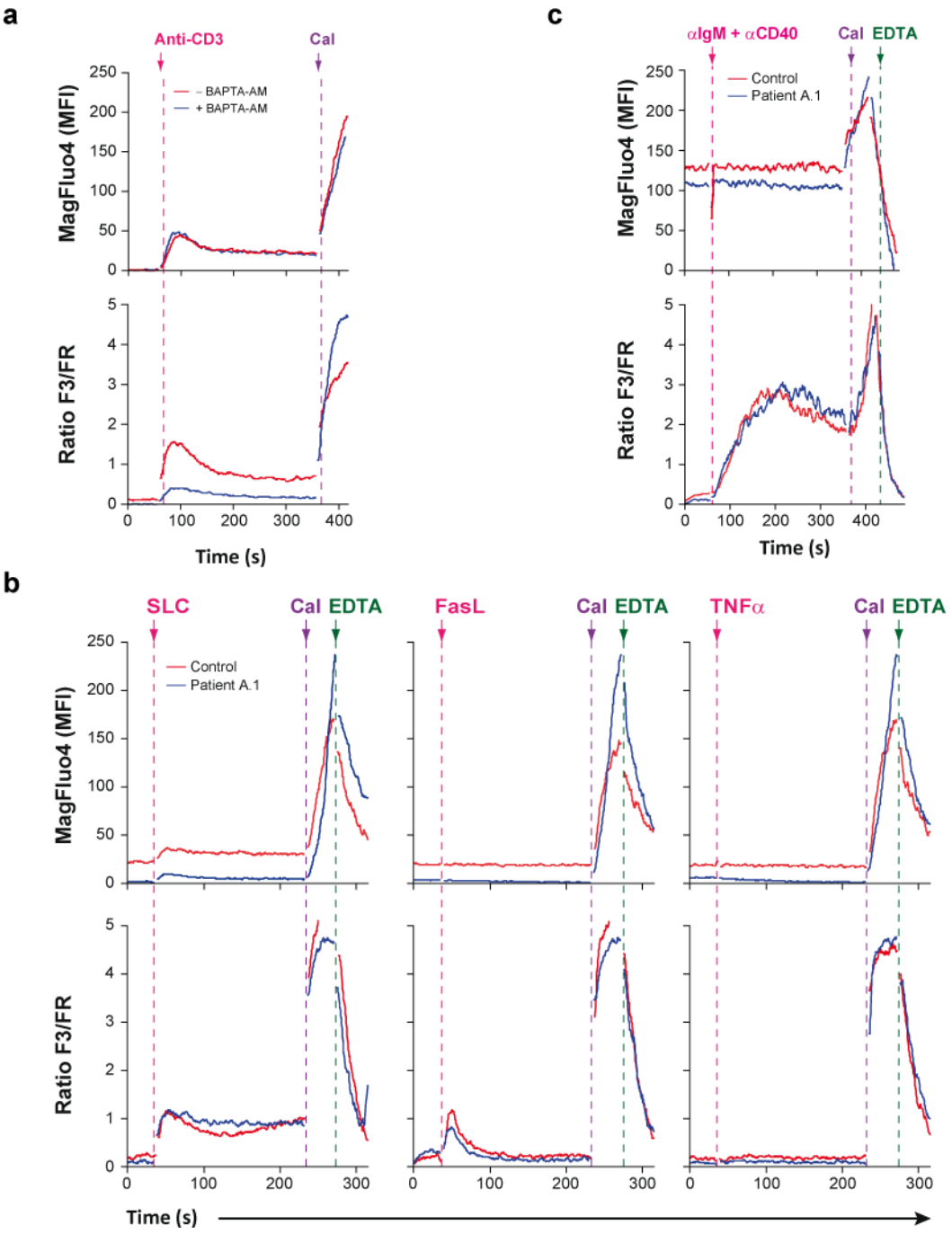
Supplementary Figure 3: Patients A.1 and A.2 have 10 bp deletion in *MAGT1* leading to altered splicing and early termination. a, Sanger sequencing of *MAGT1* genomic DNA sequence from patients A.1 and their mother. The normal allele from mother is shown on top, and shaded reads represent deleted regions in the mutant allele. **b,** PCR amplification of *MAGT1* cDNA from patients A.1 and A.2 and their parents. **c,** Sanger sequencing of *MAGT1* cDNA from patient A.1 and their mother. Expected translation from open reading frame is shown.



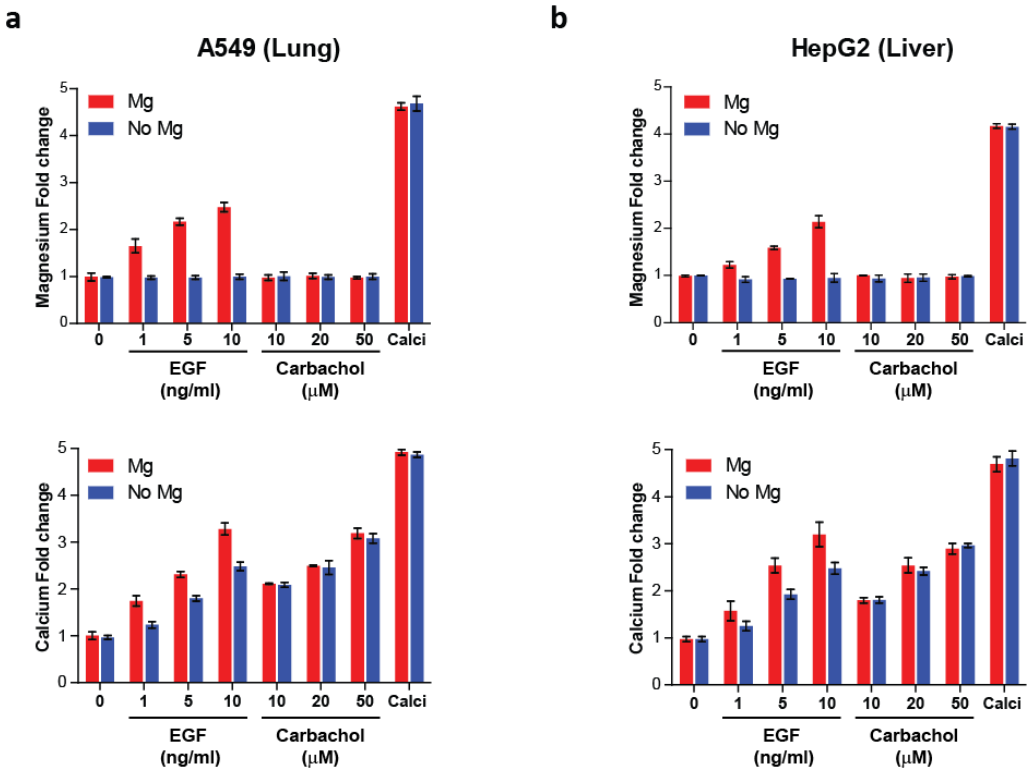
Supplementary Figure 4: Patients B.1 has a nonsense mutation in *MAGT1* and defective NF-κB and NFAT translocation. a, Sanger sequencing of *MAGT1* genomic DNA sequence from patient B.1 and normal control. The mutant nucleotide is shaded. Expected translation from open reading frame is shown. Confocal imaging of (**b**) p65 and (**c**) NFAT nuclear translocation after stimulation with anti-CD3 (1 μg/ml) in Herpes virus saimiri transformed T cells from patient B.1 or normal control. Nuclei are counterstained with Hoechst 33342 (blue). Graph represents the percentage of nuclear translocation in normal control and patient B.1. Error bars represent s.e.m. (n=3). Scale bar: 10 μm.



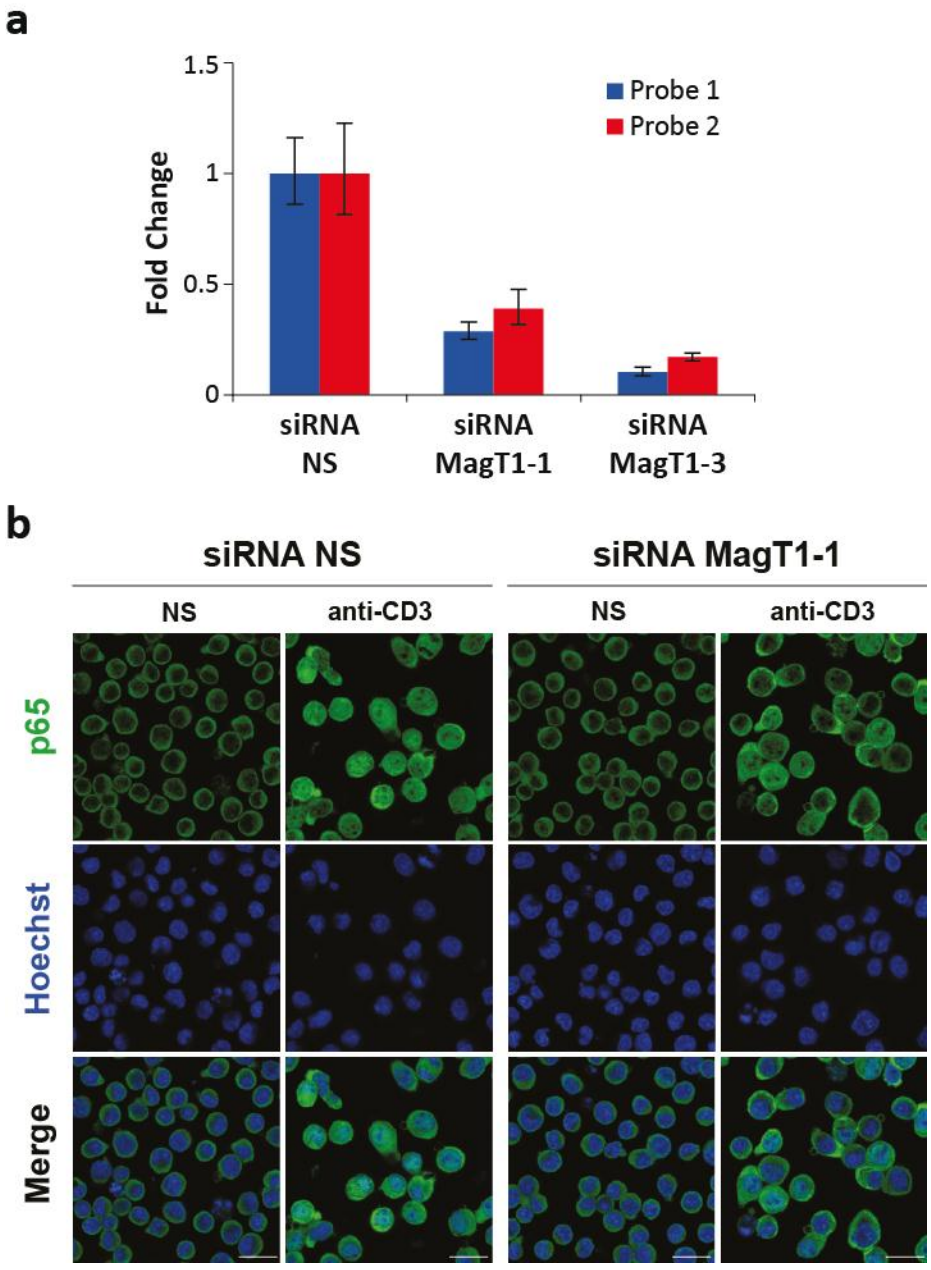
Supplementary Figure 5: Mg^{2+} uptake is defective but the total magnesium and calcium levels are not altered in T cells from patient A.1 and A.2. a, Representative flow cytometry kinetic profile of Ca^{2+} (left panel) and Mg^{2+} (right panel) uptake in T cell from normal donor or patient. T cells loaded with either Fluo3-AM/Fura Red-AM or Magfluo4-AM were incubated in a buffer without Mg^{2+} and Ca^{2+}. Baseline was recorded for 1 min. and then 1 mM Ca^{2+}, 2 mM Mg^{2+}, 5 µM of calcimycin, and 2 mM EDTA were added as indicated. **b**, Total magnesium (Mg), calcium (Ca), and sulfur (S) levels measured in activated T cells from patients and their mutation negative father by inductively coupled plasma mass spectroscopy[51]. Error bars represent s.e.m. (n=3).



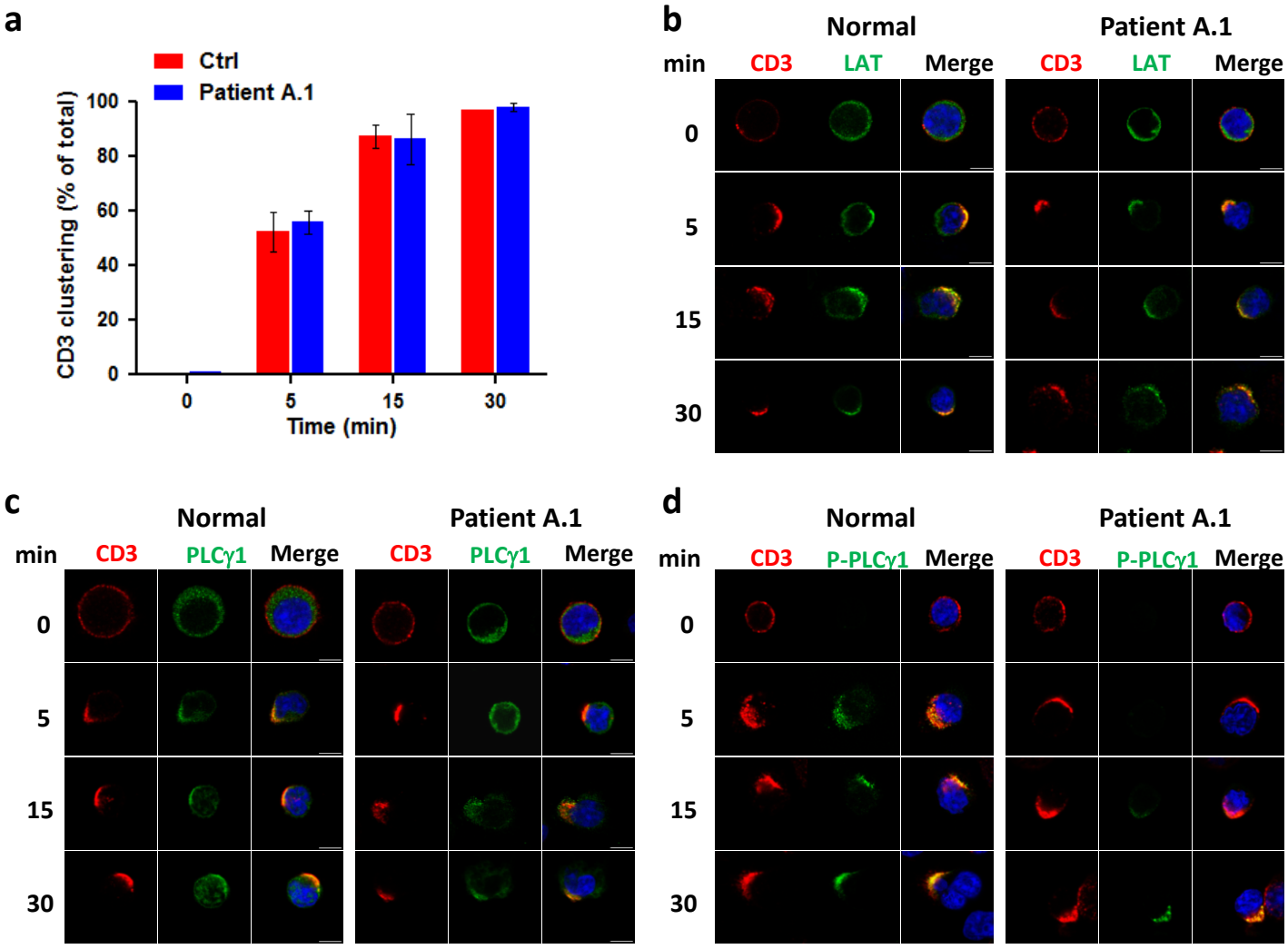
Supplementary Figure 6: Mg^{2+} influx is specific to TCR stimulation. a, Flow cytometry profile of Mg^{2+} (upper panel) and Ca^{2+} (lower panel) flux in PBMC from normal control either treated or not with 5 μM BAPTA-AM Ca^{2+} chelator. **b,** Flow cytometry kinetic profile in activated T cells from normal control or patient A1 as in **a,** stimulated with SLC/CCL21 (100 ng/ml), FasL (1 μg/ml) or TNFα (50 ng/ml). **c**, Flow cytometry kinetic profile of Mg^{2+} (upper panel) and Ca^{2+} (lower panel) flux in B cells from normal control or patient stimulated with anti-IgM (30 μg/ml) + anti-CD40 (1 μg/ml).



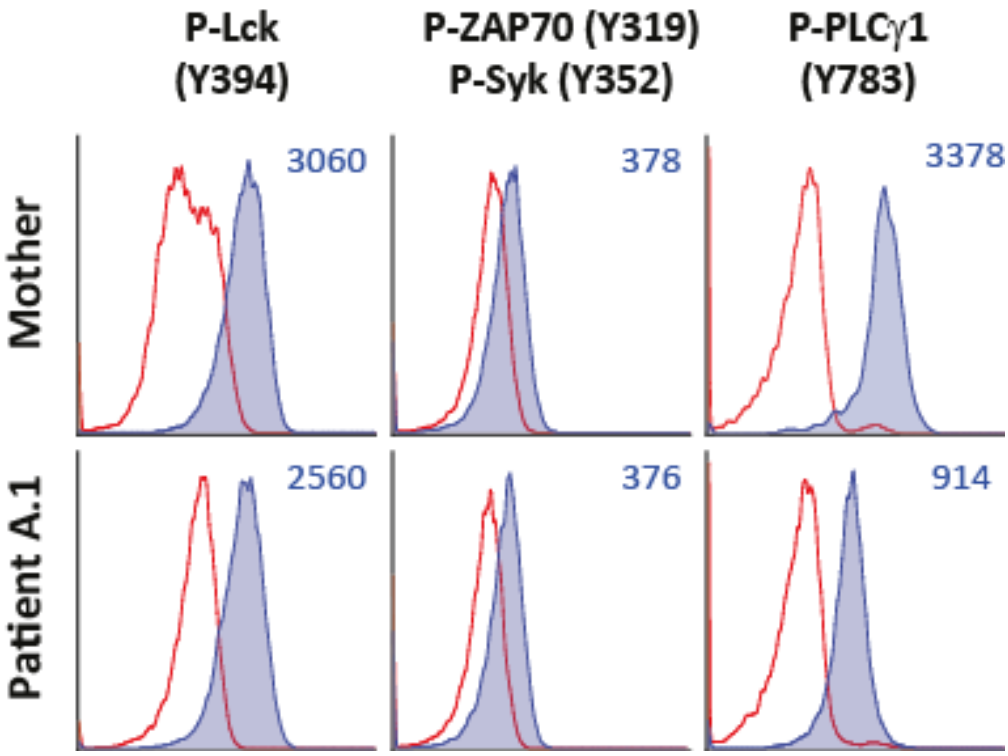
Supplementary Figure 7: Epidermal growth factor (EGF) stimulation of calcium influx in various non-immune cells requires Mg^{2+}. Histograms showing the fold change of the peak of Mg^{2+} (upper panel) and Ca^{2+} (lower panel) influxes in A549 (**a**), or HepG2 (**b**) cells stimulated with indicated concentrations of EGF, carbachol or 5 μM of calcimycin (Calci) in the presence of either 1.2 mM $MgSO_4$ or no Mg^{2+} in the extracellular fluid. Error bars represent s.e.m. (n=3).



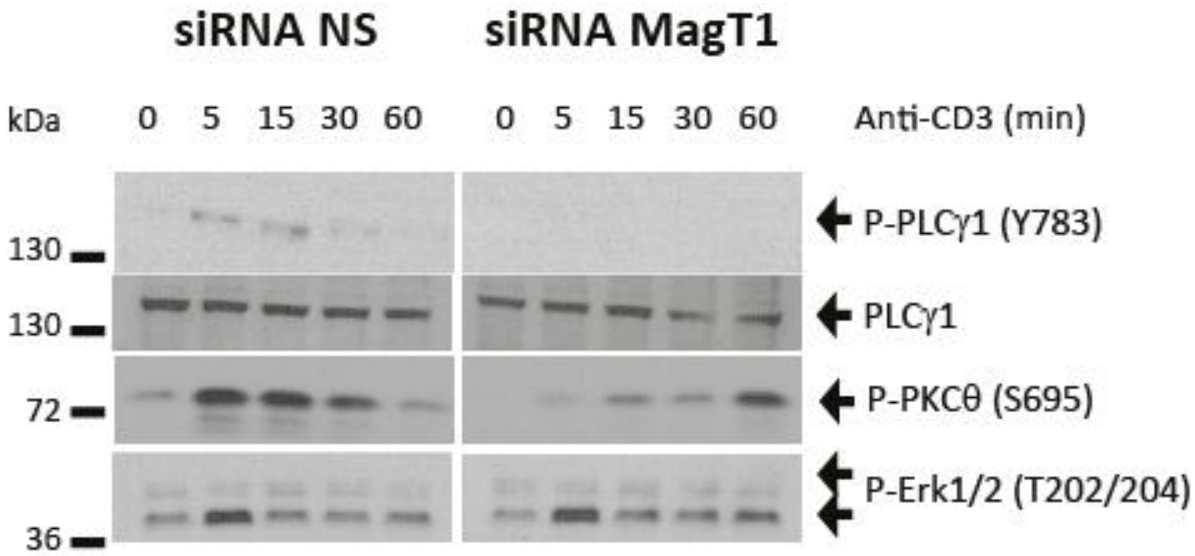
Supplementary Figure 8: MAGT1 knockdown in normal T cells recapitulates p65 nuclear translocation defect. **a,** RT-PCR assessing the efficiency of *MAGT1* mRNA knockdown. **b,** Confocal imaging of p65 nuclear translocation in knockdown cells after anti-CD3 (1 μg/ml) stimulation. Nuclei are counterstained with Hoechst 33342. (Scale bar: 10 μm).



Supplementary Figure 9: Recruitment of TCR signaling pathway components to TCR cluster. a, Quantification of cell exhibiting TCR cluster in normal and patient T cell. Error bars represent s.e.m. (n=3). Confocal images of TCR clustering together with LAT (**b**), PLCγ1 (**c**) or phospho-PLCγ1 (**d**) Nuclei were stain with Hoechst 33342. Scale bar: 5 μm. All images are representative of 3 separate experiments.



Supplementary Figure 10: MagT1 deficiency impairs PLCγ1 phosphorylation upon TCR stimulation. Flow cytometry profile of intracellular staining for phospho-Lck, phospho-ZAP70/phospho-Syk and phospho-PLCγ1 in activated T cells from normal control (mother) and patient (A.1) stimulated with 1 μg/ml anti-CD3 for 15 min. Numbers are MFIs of the stimulated cells.



Supplementary Figure 11: siRNA knockdown of MAGT1 in normal T cells recapitulates P-PLCγ1 and PKCθ defects. Total T cells purified by Pan T cell isolation kit from PBMC were transfected using P3 Primary Cell 4D-Nucleofector X kit with either Stealth RNAi Negative Control Duplexes (siRNA NS) or siRNA specifically targeting *MAGT1* (siMAGT1-3). Transfected cells were cultured in 10% RPMI media for at least 4h before stimulation with anti-CD3 and anti-CD28 antibodies (1 ug/ml each). After 48h, cells were transfected a second time and cultured 48h in 10% RPMI media supplemented with 100 U/mL rhIL-2. Cell were then starved for 4h in media without FCS and stimulated with 1 mg/ml of anti-CD3 for indicated time. Western blot analysis was performed as describe in the method section.

Supplementary Discussion

The ligand-gated Mg^{2+} influx in T cells is carried principally by MagT1, a transporter that is ubiquitously expressed and upregulated by low Mg^{2+} in human epithelial cells[36]. We therefore tested epithelial cells and found that the Ca^{2+} influx induced by EGF, but not catecholamines, is also Mg^{2+} dependent. Since EGF, like TCR, generates a Ca^{2+} signal through PLCγ1, our data indicates that Mg^{2+} acts as a second messenger that selectively increases the activation of PLCγ1 but not PLCγ2 (antigen stimulation of B cells) or PLCβ (catecholamine stimulation). The effect of Mg^{2+} is indirect because it is not required for PLCγ1 catalytic activity[54]; rather the loss of the Mg^{2+} influx causes a marked delay in the activating phosphorylation of PLCγ1. Since the recruitment of PLCγ1 is not impaired in the patients, the Mg^{2+} dependent process may either be the activation or function of ITK or a novel regulator of ITK. Alternatively, the rapid influx of Mg^{2+} might also stabilize the phosphate form nicotinic acid adenine dinucleotide, another potent stimulator of Ca^{2+} release induced by TCR engagement[4]. We have not yet uncovered abnormalities in organs outside the immune system in the MagT1 deficient patients possibly because MagT1 is functionally tissue-restricted, or because the homologous TUSC3 channel, which is poorly expressed in lymphoid tissues, substitutes for MagT1 in non-lymphoid tissue[36].

The disease manifestations of MagT1 deficient patients are characterized by decreased CD4+ T cell output and chronic viral infections, particularly by EBV. The two young patients that we initially characterized are currently still living with persistent active EBV infection, and the third patient we identified post mortem died from EBV lymphoma. Two ICL patients with inverted CD4:CD8 T cell ratios who died from EBV-related disease were previously reported[55,56]. Our patients' EBV susceptibility, along with lymphoma, T cell functional abnormalities, and dysgammaglobulinemia (presumed secondary to their T cell abnormalities), are also reminiscent of X-linked lymphoproliferative (XLP) disease due to *SH2D1A* or *BIRC4* mutations, or a similar disease due to *ITK* mutations[45]. Our identification of two unrelated families with patients exhibiting deleterious null mutations in *MAGT1* together with our functional knockdown and rescue studies strongly supports that loss of MagT1 causes this novel PID. Interestingly, the patient T cells express slightly higher levels of TCRα/β (data not shown), perhaps selected to compensate for their TCR activation defect. The selective deficiency of CD4+ T cells in XMEN patients may be due to aberrant TCR-driven thymic selection or other

Mg^{2+} or Ca^{2+} dependent developmental processes. At present, it is unclear why CD8 T cell thymic output is not affected, although these cells are functionally defective. As in ZAP70 deficient patients, total T cell functions are defective but only certain T cell lineage (CD8+ T cells) output from the thymus was affected[19,21].

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